Developmental Toxicity Models for the ToxCast Compound Library

SOL-NC-12-00024
http://www.epa.gov/oamrtpnc/1200024/index.htm
National Center for Computational Toxicology

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Office of Research and Development
National Center for Computational Toxicology
www.epa.gov/ncct

Pre-proposal Information Session, Sept 19, 2012

Computational Toxicology:
high-throughput (HTS) paradigm

- ❖ EPA's ToxCast research now providing HTS data for biological profiling >1,060 chemicals across >650 *in vitro* assays

- ❖ computational models integrate data and knowledge of target pathways and networks associated with adverse outcomes

- ❖ *in vitro* data from assays at cellular/molecular scale → predict and model the *in vivo* response at tissue/organismal scale

Current ToxCast HTS assay portfolio:

>1100 readouts / effects

Assay Provider
ACEA
Apredica
Attagene
BioSeek
CellzDirect
NCGC/Tox21
NHEERL MESC
NHEERL NeuroTox
NHEERL Zebrafish
NovaScreen
Odyssey Thera

Biological Response
cell proliferation and death
cell differentiation
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor activity
receptor binding

Target Family
Response Element
Transporter
Cytokines
Kinases
Nuclear Receptor
CYP450 / ADME
Cholinesterase
Phosphatases
Proteases
XME metabolism
GPCRs
Ion Channels

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type
Single
Multiplexed
Multiparametric

Cell Format
Cell free
Cell lines
Primary cells
Complex cultures
Free-living embryos

Species
Human
Rat
Mouse
Zebrafish
Sheep
Boar
Rabbit
Cattle
Guinea pig

Tissue Source
Lung   Breast
Liver   Vascular
Skin   Kidney
Cervix   Testis
Uterus   Brain
Intestinal   Spleen
Bladder   Ovary
Pancreas   Prostate
Inflammatory Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrasyscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

List of assays and related information at: **http://www.epa.gov/ncct/**

1st generation ToxCast predictive models

❖ **Endpoint-based models**

    liver tumors: Judson et al. 2010, Env Hlth Persp 118: 485-492

    hepatocarcinogenesis: Shah et al. 2011, PLoS One 6(2): e14584

    hallmarks of cancer: Kleinstreuer et al. 2012, submitted

    rat fertility: Martin et al. 2011, Biol Reprod 85: 327-339

    rat-rabbit prenatal devtox: Sipes et al. 2011, Toxicol Sci 124: 109-127

    zebrafish vs ToxRefDB: Sipes et al. 2011, Birth Defects Res C 93: 256-267

❖ **Pathway-based models**

    endocrine disruption: Reif et al. 2010, Env Hlth Persp 118: 1714-1720

    microdosimetry: Wambaugh and Shah 2010, PLoS Comp Biol 6: e1000756

    mESC differentiation: Chandler et al. 2011, PLoS One 6(6): e18540

    hESC metabolomics: Kleinstreuer et al. 2011, Toxicol Appl Pharm 257: 111-121

    HTP risk assessment: Judson et al. 2011, Chem Res Toxicol 24: 451-462

    angiogenesis: Kleinstreuer et al. 2011, Env Hlth Persp 119: 1596-1603

Complete list of publications at: **http://www.epa.gov/ncct/**

Prediction model: prenatal developmental toxicity

Rat Model
71% balanced accuracy

Feature	Description	Weight
RAR	Retinoic Acid receptor	0.58
GPCR	G-Protein-Coupled Receptors	0.55
TGFβ	Transforming Growth Factor β	0.38
MT	Microtubule organization	0.30
SENS_CYP	Cytochrome P450 (sensitive)	0.26
AP1	Activator protein 1	0.24
SLCO1B1	Organic anion transporter 1B1	0.11
CYP	CYPs (other)	0.06
HLA-DR	MHC complex	-0.38
PXR	Pregnane X receptor	-0.24
IL8	Interleukin 8	-0.23
PGE2	Prostaglandin E2 response	-0.18

Rabbit Model
74% balanced accuracy

Feature	Description	Weight
CCL2	Chemokine ligand 2 (MCP1)	1.15
IL	Interleukin (1a and 8)	0.39
CYP	Cytochrome P450	0.24
TGFβ	Transforming Growth Factor β	0.28
MESC	Mouse ES cells (J1)	0.13
SULT2A1	Sulfotransferase	-0.26
PGE2	Prostaglandin E2 response	-0.15

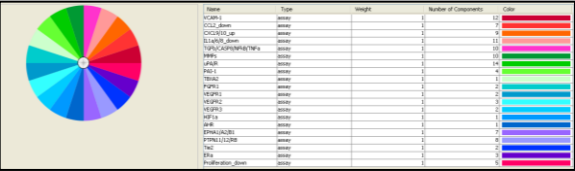*GO BIOLOGICAL PROCESS*
Feature Relations Map
⭐ *angiogenesis*
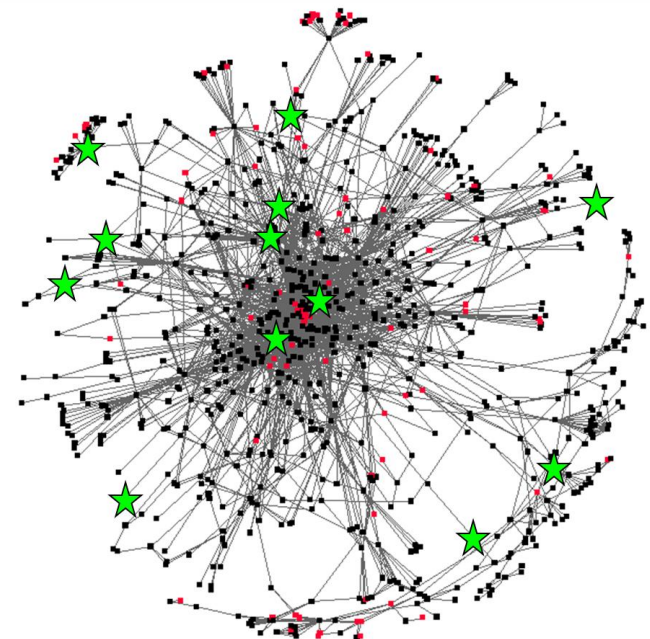
Many features can be mapped to vascular development (pVDCs)
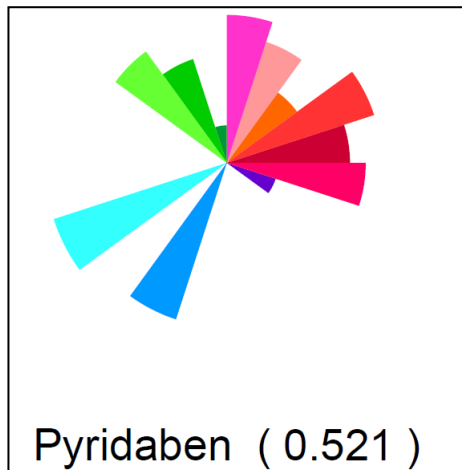
5

pVDC ranking: 1060 compounds

Thalidomide analogue
- disrupts vascular development
5HPP−33 (0.591)

Mitocide/insecticide
- mitochondrial respiratory chain
Pyridaben (0.521)
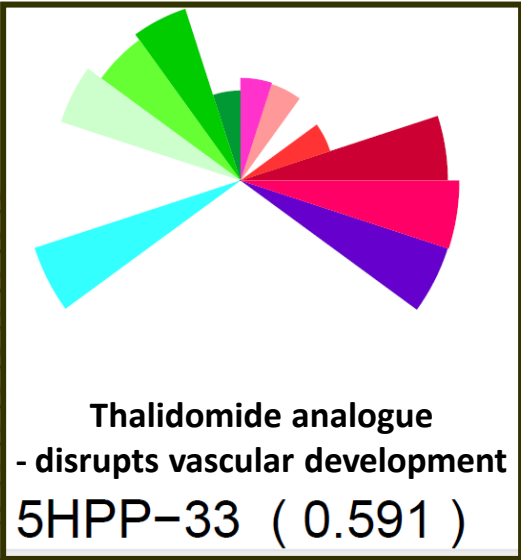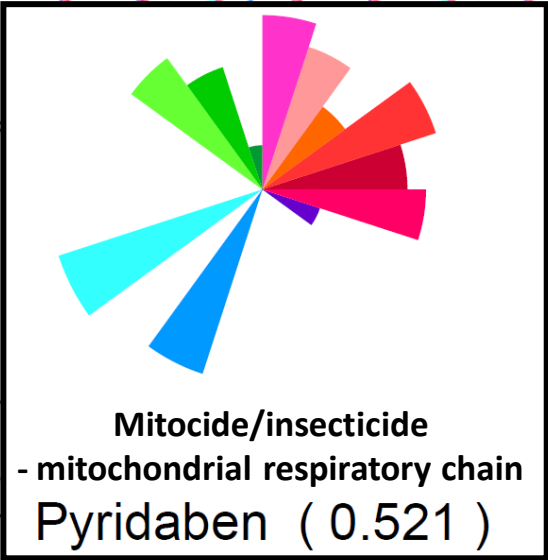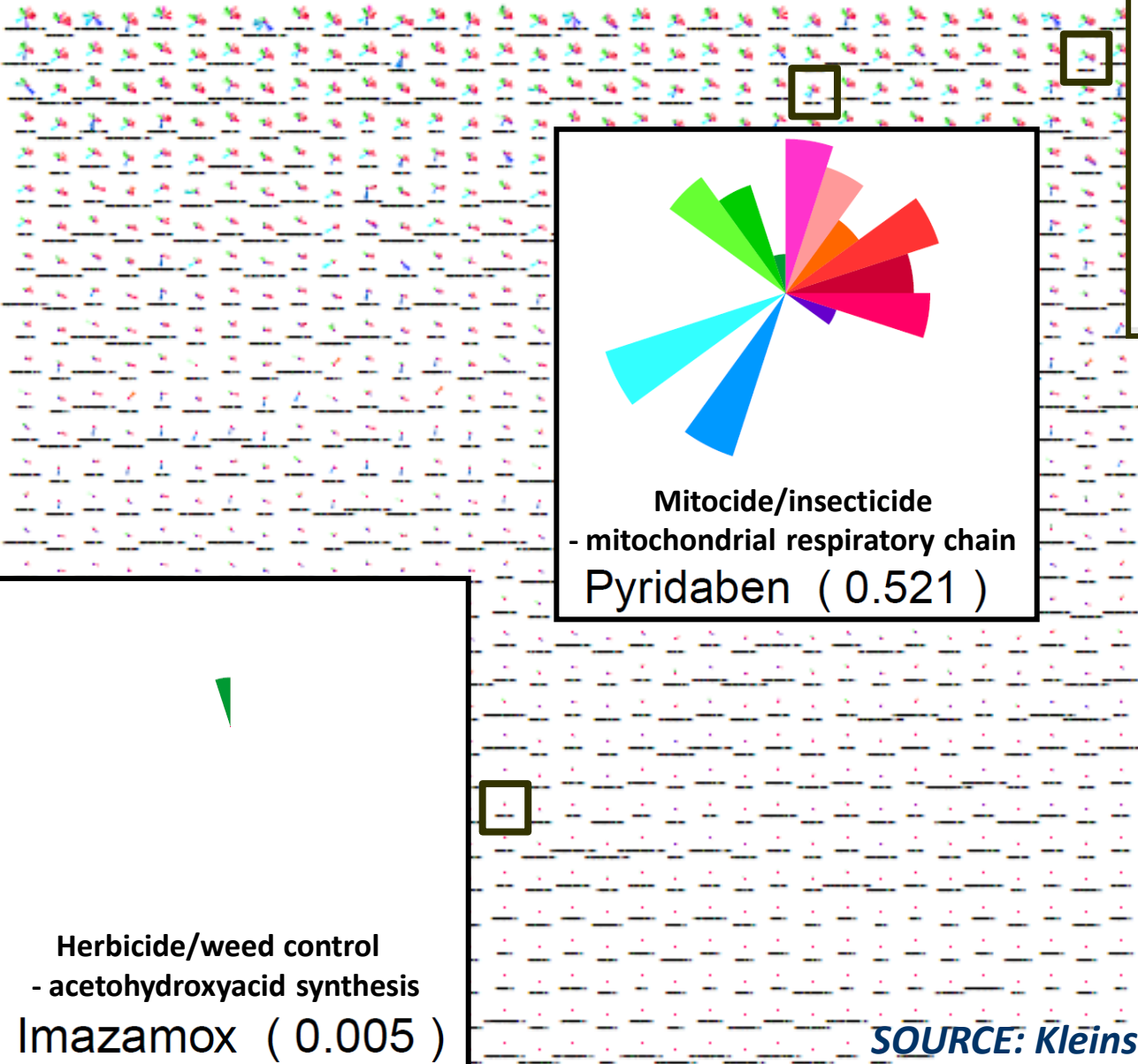
ToxPi for vascular development

Herbicide/weed control
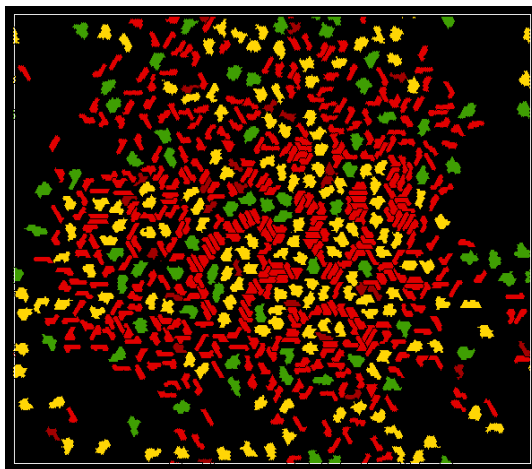- acetohydroxyacid synthesis
Imazamox (0.005)

SOURCE: Kleinstreuer et al. (in preparation)
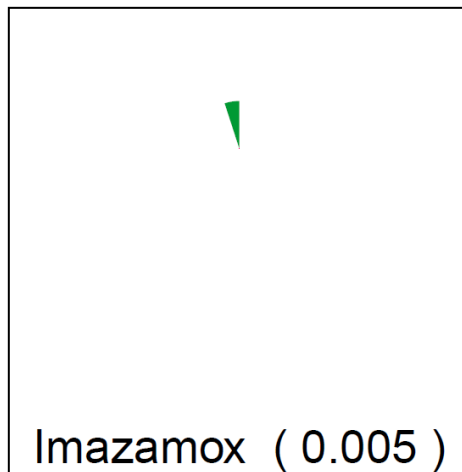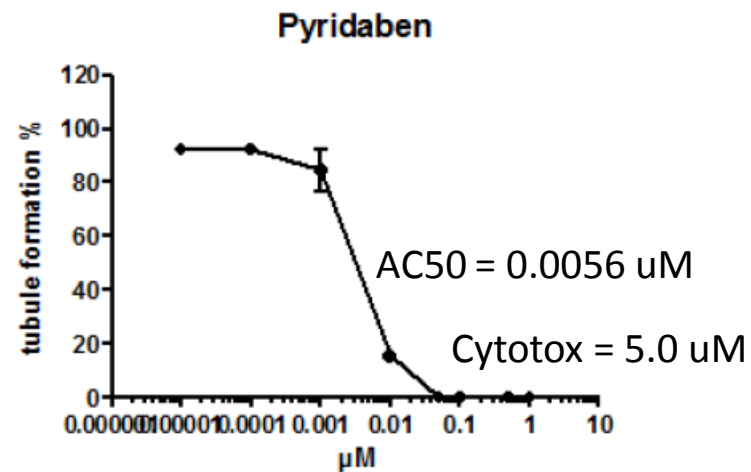
Preliminary result: vasculogenesis

ToxCast prediction	VT simulation	In vitro qualification

Pyridaben (0.521)

Pyridaben

AC50 = 0.0056 uM

Cytotox = 5.0 uM

Imazamox (0.005)

Imazamox

AC50 = 8109 uM

stimulatory
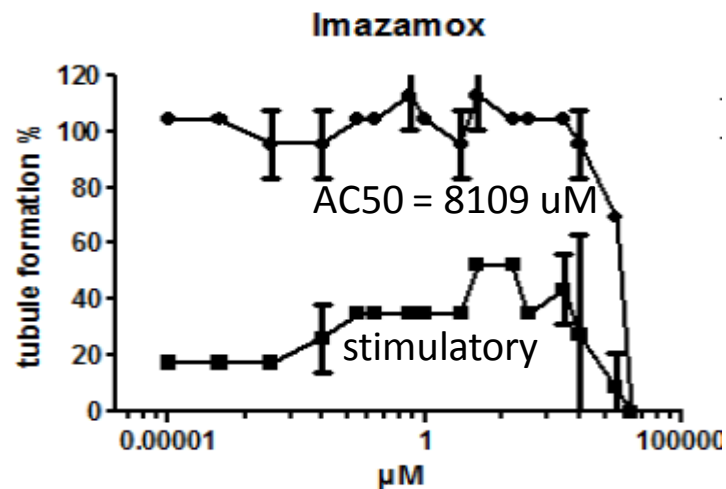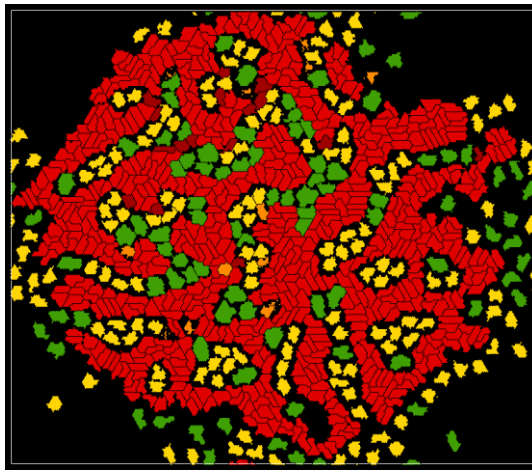
Preliminary result: VEGF-EGFP transgenic zebrafish

DMSO

0.17 μM PTK787

0.98 μM PTK787

1.8 μM PTK787
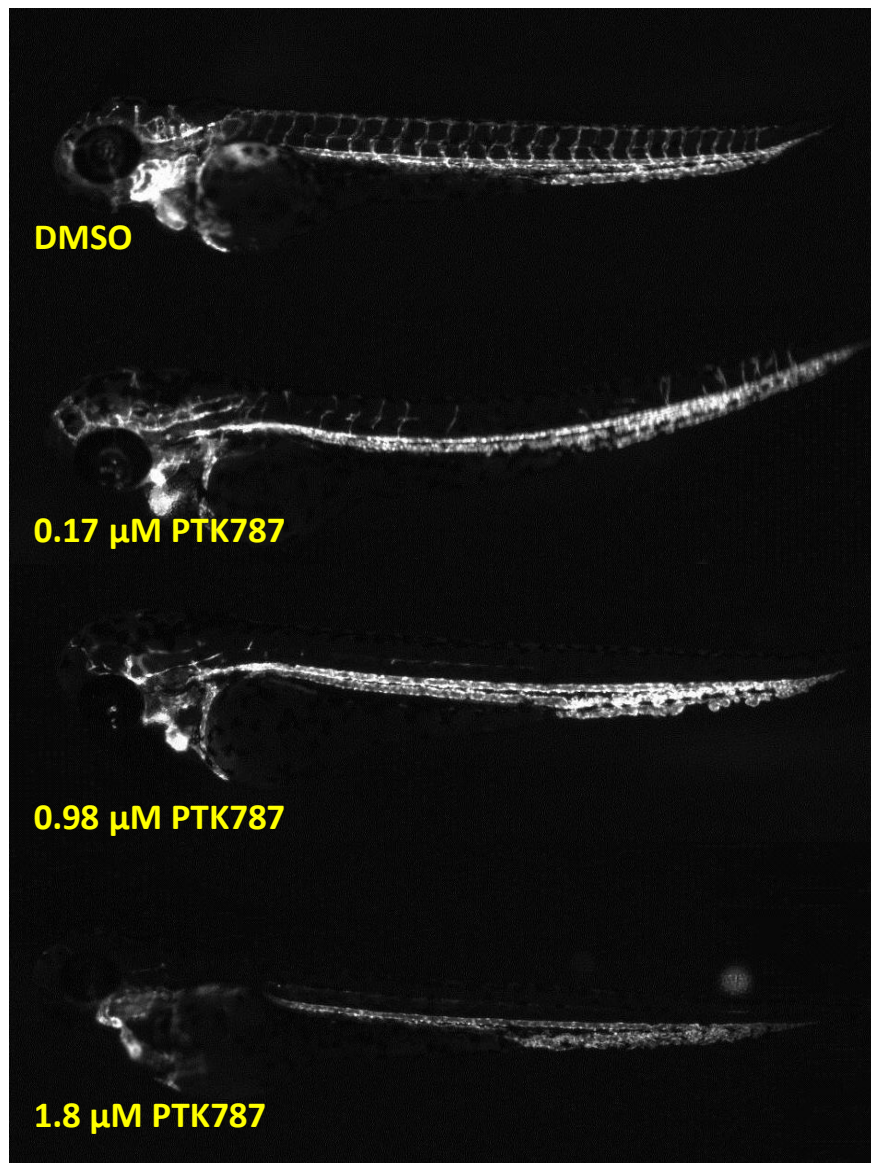
■ ToxCast pVDC (predicted positive)
■ ToxCast non-pVDC (predicted negative)

pVDC	angiodysplasia	LEC
Pyridaben	spacey caudal vein	<1 uM
Diniconazole	spacey caudal vein	8 uM
Tobupirimifos	spacey caudal vein	40 uM
S-Bioallethrin	vascular dysmorph	0.03 uM
Rotenone	vascular dysmorph	0.03 uM
Pyraclostrobin	vascular dysmorph	0.25 uM
Trifloxystrobin	vascular disruption	0.35 uM
Imazapyr	no vascular phenotype	--
Imazalil	no vascular phenotype	--
Pymetrozin	no vascular phenotype	--

SOURCE: T Tal, S Padilla – EPA/NHEERL
C McCollum, M Bondesson – U Houston

A developmental AOP: children of thalidomide
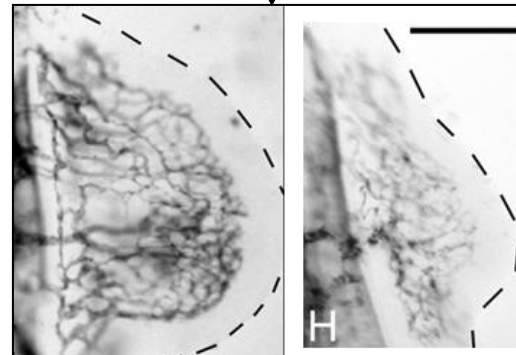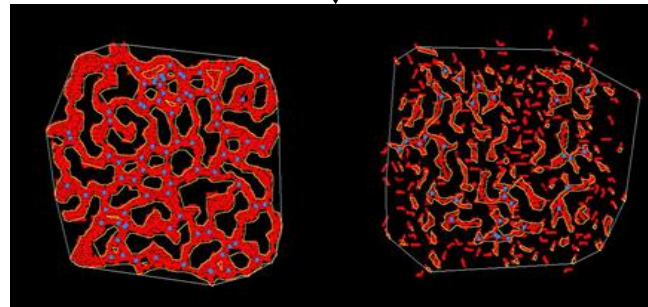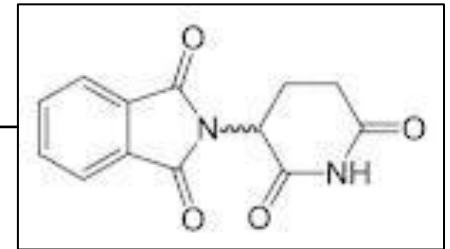
CRBN
cereblon (proteasome)

cell-cell signaling
molecular (FGF) gradients
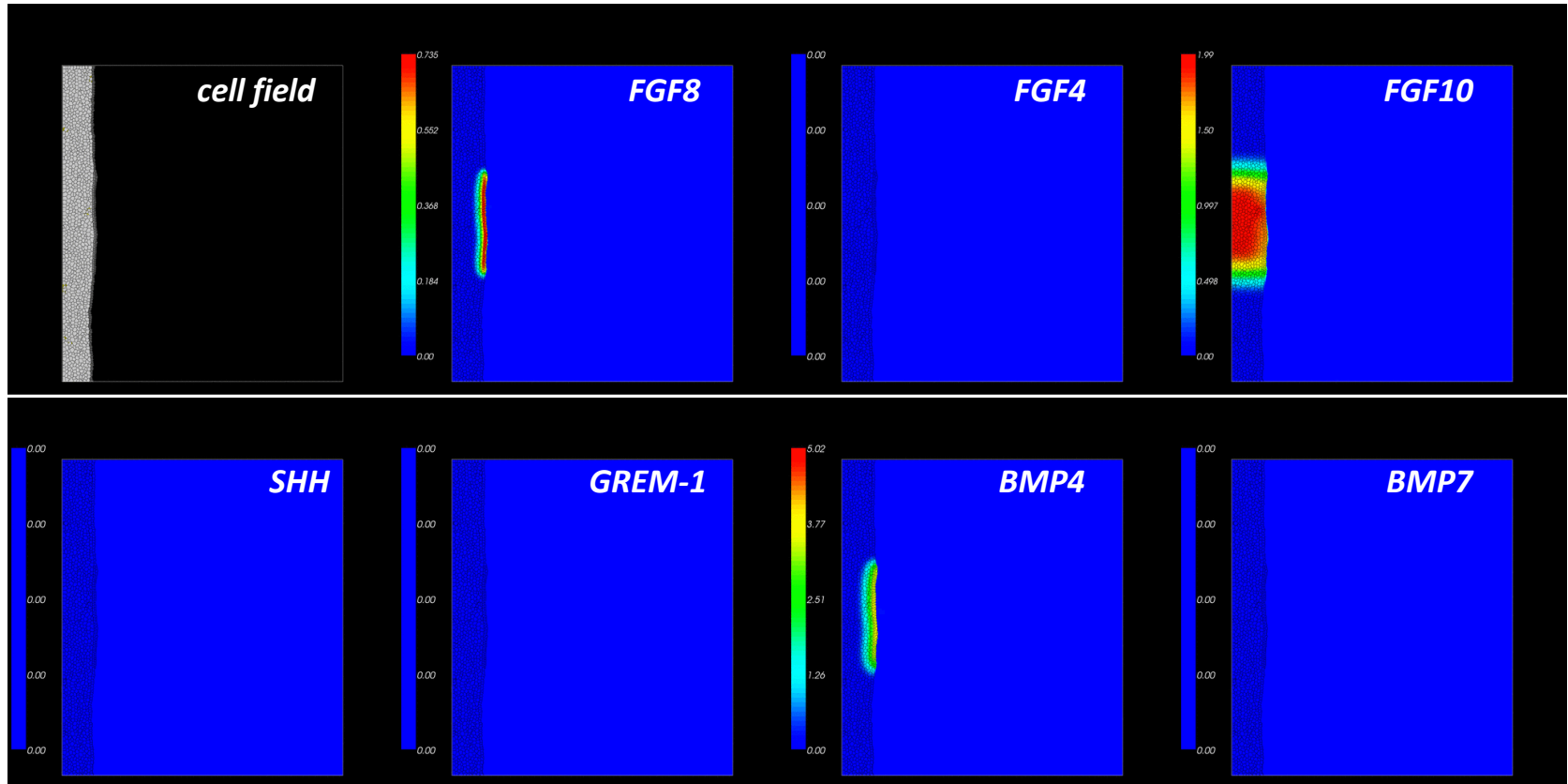
cellular behaviors
pathway dysregulation

embryonic vasculature
vascular disruption

early limb-buds
disruption of outgrowth
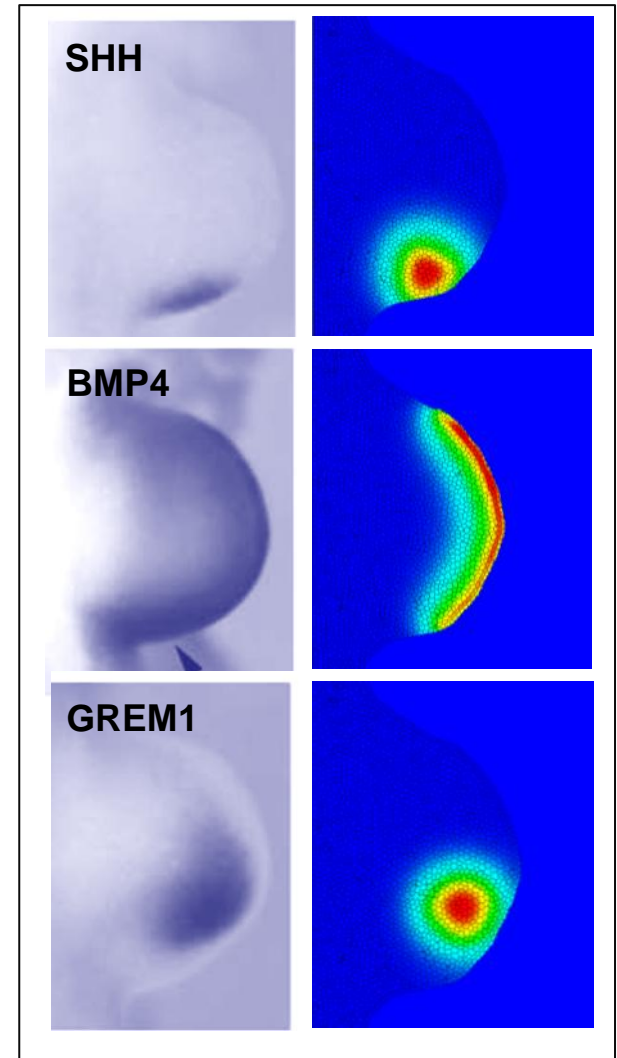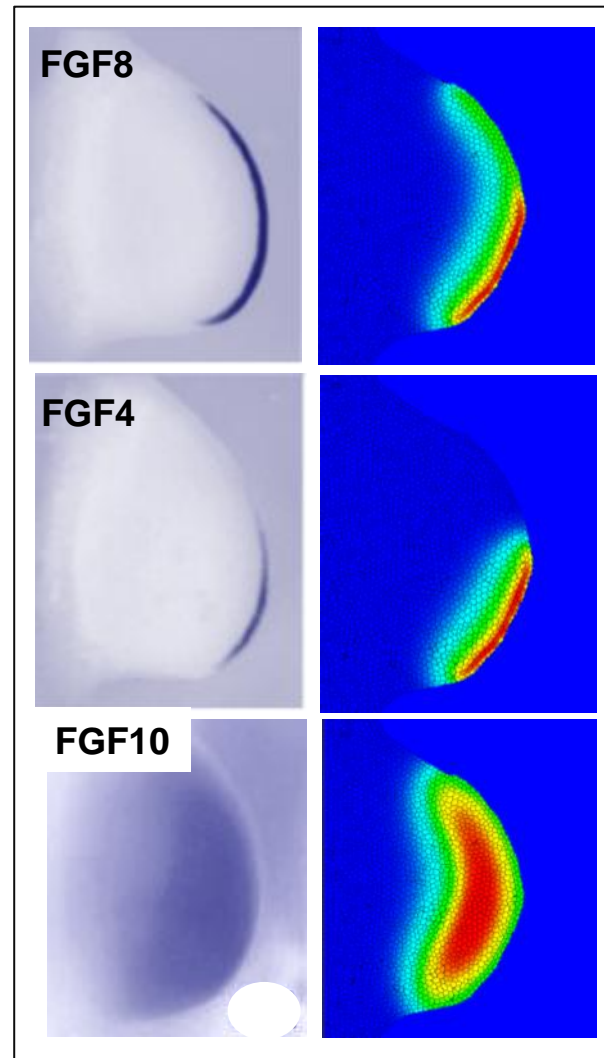
birth defects
limb malformations

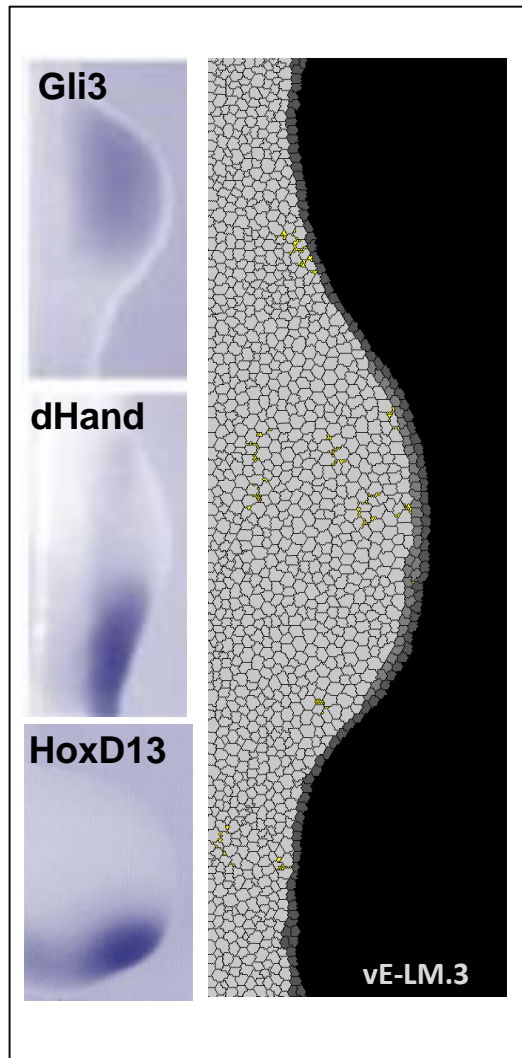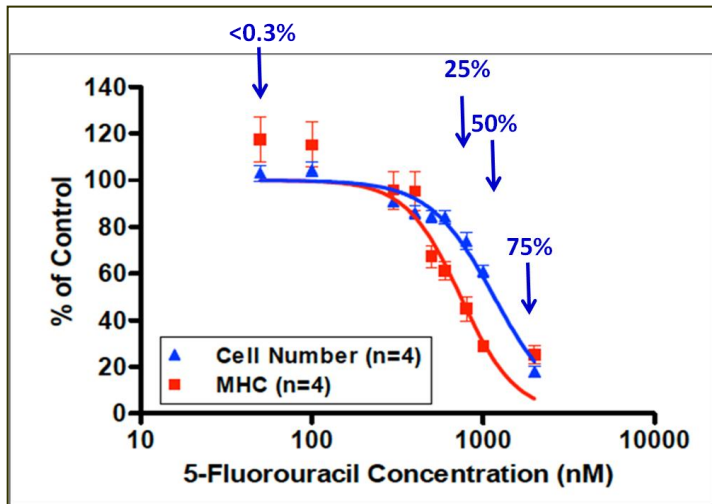# Virtual Embryo – limb development module

vE-LM.3 (48,000 MCS @ 1,000 MCS/hr)

Virtual Embryo – limb development module

ISH (mouse literature) vs vE-LM.3 at 40K MCS

# **Simulating chemical injury:** excessive cell death

mESC data translated into dynamic simulation

Responsiveness to SOL-NC-12-00024:

❖ Assays to determine the effects of ToxCast compounds for features relevant to developmental processes and toxicities.

❖ Features should relate directly to morphological defects that manifest during prenatal or early postnatal life.

❖ Parameters should provide broad coverage of metabolic and/or regulatory pathways important for embryogenesis or early postnatal development.

❖ Potential pathway-level targets leading to developmental defects relevant to the human condition.

❖ Multiple awards could be made to cover the scope and breadth of developmental pathways, processes, and toxicities.

# EPA's Virtual Embryo

http://www.epa.gov/ncct/v-Embryo/
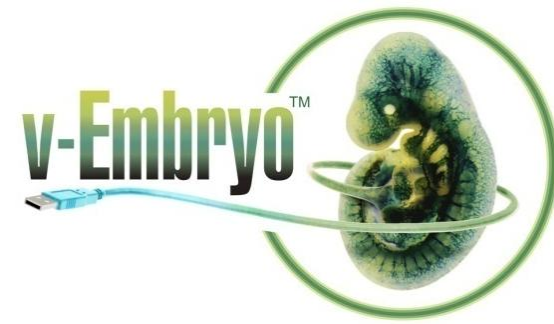
❖ Data needs to support computational models for predictive toxicology in developing systems include, but are not necessarily limited to assay platforms that:

1. generate relevant data to enhance predictive models of developmental toxicity, expanding the ToxCast assay portfolio.

2. confirm or qualify results of the predictions and provide mechanistic support for model assessment.

Assay technologies might include, for example:

❖ stem cell (ES, iPS) growth and differentiation
  - *PATHWAYS & PROCESSES*
  - *SELF-RENEWAL & PLURIPOTENCY*
  - *LINEAGE DETERMINATION & DIFFERENTIATION*
  - *GENOMICS & EPIGENOMICS*
  - *METABOLOMICS*
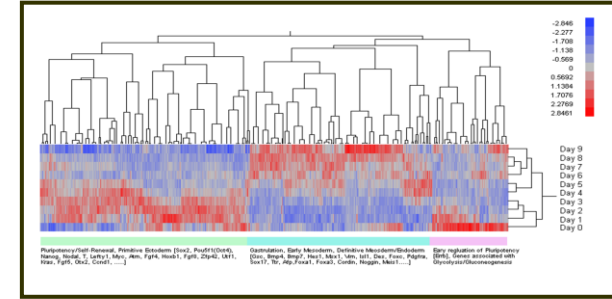
❖ small model organisms such as ZFE
  - *CELLULAR NETWORKS & CELLULAR IMAGING*
  - *LESION PROPAGATION & DYSMORPHOGENESIS*
  - *MORPHOGENETIC INTERACTIONS & MOVEMENTS*
  - *GENOMICS & EPIGENOMICS*
  - *METABOLOMICS*
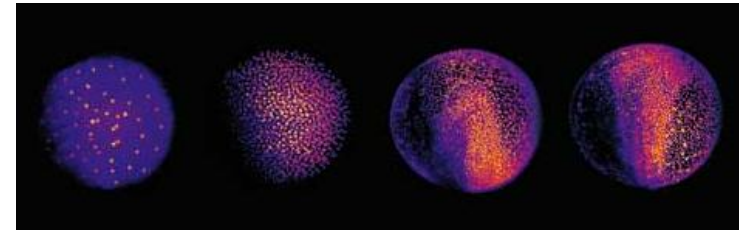
❖ embryological and 3D culture models
  - *TISSUE FUSION & HETEROTYPIC INTERACTIONS*
  - *IMMUNOHISTOCHEMISTRY & IN SITU HYBRIDIZATION*
  - *GENOMICS & EPIGENOMICS*
  - *METABOLOMICS*